Nitrification☆

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Introduction

Nitrification is an essential process in the nitrogen cycle of soils, natural waters, and wastewater treatment systems. It is responsible for the biological conversion of ammonium to nitrate. While both of these compounds are suitable for plant use as nutrients, they behave quite differently in soil systems, and have quite different sources and fates in the marine environment. Ammonium is produced as a waste product from cellular and organismal metabolism, a breakdown product of organic material. It is the preferred nitrogen source for many plants and algae. Nitrate is not only a nutrient, but the substrate for the bacterial process of denitrification, by which nitrate is reduced to dinitrogen gas, N_2. Most plants cannot use dinitrogen gas as a nitrogen source, so denitrification represents a loss term for fixed nitrogen in the ecosystem. Nitrification itself does not directly affect the nitrogen budget, but by linking organic matter decomposition to denitrification, it completes the N cycle.

The significance of nitrification can be summarized in the following list, and the individual items are described in the sections below: (1) transformation of ammonium to nitrate, with implications for the availability of N for plants and algae, (2) production of substrate for denitrification, (3) production of nitrous oxide in aquatic and terrestrial ecosystems, (4) consumption of oxygen in sediments, (5) acidification of the environment.

Nitrifying Microorganisms

Ammonia-Oxidizing Bacteria

Nitrification is performed by two functionally defined groups of microbes, referred to together as nitrifiers. The first group of nitrifiers is the ammonia oxidizers, which oxidize ammonia to nitrite. In most natural waters, ammonium is present predominantly as the positively charged ion, ammonium (NH_4^+), but the enzyme responsible for the first step of the reaction uses the gaseous form, NH_3, which is usually a minor component at equilibrium. We shall use the term ammonium when we are mainly concerned with the form that is important in the environment, and ammonia when referring to the enzymatic oxidation process of the specific substrate. There are two very different groups of ammonia-oxidizing microbes. One is the well-known bacterial group (ammonia-oxidizing bacteria, AOB), which includes a few different kinds of bacteria that all make a living by generating reducing power (ATP) from the oxidation of ammonia and using that energy to fix carbon dioxide (Bock and Wagner, 2006). They are generally considered to be obligate autotrophs, that is, they are unable to utilize or grow on organic carbon to any important extent, and can grow only by fixing their own CO_2 using the Calvin cycle. Ammonia is their only energy source, and their main metabolic

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product is nitrite. Nitrous oxide is a minor product of ammonia oxidation, and is produced by two different pathways. AOB have been cultivated for over 100 years and their description played an important role in the discovery and early research on chemoautotrophy.

Ammonia-Oxidizing Archaea

A second distinct group of ammonia-oxidizing microbes has only recently been recognized and brought into culture only in 2005 (Konneke et al., 2005). These are not bacteria, but archaea (ammonia-oxidizing archaea, AOA). Like AOB, AOA oxidize ammonia to nitrite and produce nitrous oxide and nitrite from ammonia, but the enzymatic pathways are quite different. AOA are also thought to be predominantly autotrophic, but they fix CO_2 using the 3-hydroxypropionate/4-hydroxybutyrate pathway, rather than the Calvin Cycle (Walker et al., 2010). AOA are abundant in many environments and in the ocean and many terrestrial systems, they far outnumber the AOB. In estuaries, the ratio of AOB to AOB varies widely, with AOB sometimes more abundant.

Although the enzymes and pathways differ for the AOA and AOB, aerobic ammonia oxidation in both groups apparently proceeds by the same stoichiometry:

$$NH_3 + 1.5\,O_2 \rightarrow NO_2^- + H_2O + H^+$$

In addition to the net production of nitrite by the above equation, AOB are also capable of producing nitrous oxide (N_2O) by two distinct pathways. Most AOB investigated to date possess the genes and enzymes necessary for the partial denitrification pathway that reduces nitrite to nitric oxide (NO) and then to N_2O (Casciotti and Ward, 2001, Casciotti and Ward, 2005). The genes involved are homologous to those found in denitrifiers, and the process is often referred to as nitrifier denitrification. The result is the production of N_2O, whereas complete denitrification by the usual denitrifying bacteria produces N_2O only as a transient intermediate. A second pathway produces N_2O from hydroxylamine (NH_2OH), which is an intermediate in the oxidation of ammonia to nitrite by the AOB. The nitrifier denitrification pathway of N_2O production is favored during nitrification at low oxygen concentrations, implying that nitrifiers use this pathway for anaerobic respiration, just as in denitrifiers. AOA also produce N_2O, in approximately the same proportion to NO_2 as in AOB, and with similar isotopic fractionation (Santoro et al., 2011). Nevertheless, the pathways of N_2O production in AOA are unknown, but almost certainly are different from the pathways in AOB. Significantly, AOA do not use NH_2OH as an intermediate, so the production of N_2O from NH_2OH cannot occur in AOA, and AOA do not possess the reductive enzymes used in nitrifier denitrification by AOB.

Anammox Organisms

A third group of bacteria, members of the Planctomycetes phylum, are capable of oxidizing ammonium using nitrite instead of oxygen and producing N_2 instead of nitrite (Kuenen, 2008). This metabolism is strictly anoxic and the process is known as anaerobic ammonia oxidation, or anammox. Anammox organisms are unique in a number of ways; the pathway for oxidation of ammonium is not very similar to that found in AOB or AOA, although the enzymes involved may be evolutionarily related. Hydrazine, more commonly associated with rocket fuel than with biological systems, is an intermediate, while hydroxylamine, an intermediate in AOB, is apparently not involved in anammox. Anammox organisms are strict autotrophs, and apparently use the acetyl-CoA pathway for CO_2 fixation. Their growth is extremely slow, with generation times on the order of 2 weeks. The cells contain an internal membrane-bound 'organelle' called the anammoxosome, in which the anammox reaction is localized. The cell membranes contain unique lipids called ladderanes, after their diagrammatic appearance as a ladder, which form the very dense membrane needed to handle hydrazine as an intermediate, and to prevent its diffusion out of the anammoxosome (van Niftrik and Jetten, 2008). The net reaction for anammox involves a 1:1 combination of ammonium and nitrite in the production of N_2.

$$NO_2^- + NH_4^+ \rightarrow N_2 + 2H_2O$$

Thus, unlike conventional nitrification, anammox results in the loss of fixed nitrogen from the system, and is ecologically equivalent to denitrification, rather than to nitrification. Anammox results in the anaerobic removal of ammonium using nitrite, derived from either aerobic ammonium oxidation or partial denitrification, as the oxidant.

Nitrite-Oxidizing Bacteria

The second functionally defined group of nitrifying microbes is the nitrite-oxidizing bacteria (NOB), which include several genera. The best-known cultivated members, in the genus *Nitrobacter*, are chemolithoautotrophic, like the AOB, using nitrite as an energy source and CO_2 as a carbon source via the Calvin cycle (Bock and Wagner, 2006). However, the lesser know genus, *Nitrospina*, is apparently most abundant in the ocean, and uses the reductive tricarboxylic acid pathway for CO_2 fixation. Many strains are known to possess heterotrophic capabilities and are considered mixotrophic or facultative autotrophs. Although they have limited metabolic capabilities for uptake and degradation of organic molecules, they can supplement their growth with organic carbon and, in some cases, grow slowly in the absence of nitrite when certain organic substrates are present. The oxidation of nitrite is even less energy yielding than ammonia oxidation, so perhaps this ability for heterotrophic growth is not surprising. Aerobic nitrite oxidation proceeds by the following stoichiometry:

$$NO_2^- + 0.5O_2 \rightarrow NO_3^-$$

There are no other pathways, nor any different kinds of bacteria or archaea known to be capable of or involved in nitrite oxidation in the environment. The recent finding of greater diversity among ammonia-oxidizing microbes begs the question, however, of whether additional nitrite oxidation pathways and organisms remain to be discovered.

Heterotrophic Nitrifiers

The ability to nitrify, via pathways involving the inorganic transformations normally associated with the autotrophic nitrifiers described above, or via pathways involving organic intermediates but resulting in the net oxidation of ammonium, has been attributed to some heterotrophic bacteria and fungi. Heterotrophic nitrification does not conserve energy (i.e., is not linked to ATP production) and the rates observed are much slower than rates found in cultivated conventional nitrifiers. Autotrophic nitrifiers are susceptible to inhibition by a number of naturally occurring substances, including secondary metabolites of some trees, for example. AOB are inhibited by acidic conditions, which pertain in some soils. These observations led to the suggestions that heterotrophic nitrification might be particularly important under conditions in some soils that are very unfavorable for known autotrophic nitrifiers. The quantitative importance of heterotrophic nitrification remains uncertain in both aquatic and terrestrial environments.

Ecological Roles of Nitrification

Agricultural and Terrestrial Systems

Nitrogen is the main component of fertilizers applied in many agricultural systems. Addition of N as ammonium is advantageous because it is easily assimilated by plants and, due to its positive charge, it binds to soil particles and is somewhat resistant to loss in runoff. Nitrifying bacteria in the soil can convert the ammonium to nitrate, which is more easily lost in the soil solution, thus reducing the efficiency and increasing the cost of fertilizer application. Nitrification inhibitors are therefore often applied along with fertilizers, to slow down this conversion and increase the amount of N available to the plants.

Not only is the nitrate more susceptible to physical loss from the system, but it is also the substrate for denitrification. If soils become waterlogged to the extent that interstitial spaces become anoxic, denitrifying bacteria present in the soils will switch to anaerobic metabolism, in which they respire oxides of nitrogen, beginning with nitrate, instead of oxygen. Nitrate respiration leads to the removal of nitrate by its conversion to N_2 gas, which is not biologically available to most plants and is lost from the system by evasion. Both ammonia-oxidizing and denitrifying bacteria can carry out the reduction of nitrite to N_2O. For denitrifiers, this is part of the usual pathway from nitrate to N_2 ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$). For AOB, the pathway is analogous but includes only the steps $NO_2^- \rightarrow NO \rightarrow N_2O$ and it is not known what purpose it serves. Most of the N_2O produced by ammonia oxidation is probably produced by AOA via a so far undescribed pathway (Santoro et al., 2011). Especially in low oxygen conditions, substantial nitrogen can be lost as N_2O. Not only is this N lost from the bioavailable pool, but it plays a very important role in the atmosphere as a greenhouse gas. N_2O has a radiative forcing that is on the order of 200 times more potent per molecule than CO_2, the most abundant greenhouse gas. Thus, N_2O fluxes from agricultural systems to the atmosphere are potentially of concern. Management practices that optimize the amount of N added, and the timing and amount of water applied, are important in minimizing N loss both as N_2 and N_2O. Inhibition of nitrification is also widely seen as the main point of control for N loss from agricultural systems, and is practiced by the application of various commercial additives with varying degrees of specificity for nitrification over other microbial processes.

Wastewater Treatment

Waste nitrogen enters the wastewater stream in the form of ammonia, urea, and organic nitrogen. The first steps in wastewater treatment involve oxidative degradation of organic matter, resulting in the conversion of dissolved organic N into ammonium. If released as effluent in this form, the ammonium would fertilize the receiving waters, potentially leading to eutrophication and the growth of undesirable plant life and eventually possibly anoxia. Tertiary wastewater treatment is designed to remove inorganic nitrogen from the stream by nitrification. When carried out by AOB and AOA, this is an obligately aerobic process, and requires aeration to allow the growth and activity of AOB, AOA and NOB to produce nitrate. Water containing the nitrate thus produced is then subjected to an anoxic treatment in which denitrification reduces the nitrate to N_2. In this form, the effluent does not increase the bioavailable N in the receiving waters. Optimization of nitrification in wastewater treatment is the subject of much research, focusing both on the species composition of nitrifying communities in wastewater systems and biofilms, and on the physiology of nitrifiers subjected to the many potentially inhibitory components of wastewater.

Tertiary treatment is an expensive component of wastewater treatment, especially as it involves the cultivation of fastidious microbes and the sequential use of oxic, then anoxic conditions, usually requiring multiple tanks and pumping systems. It is clear, therefore, why the discovery of anammox led to its immediate patenting and a flurry of study on the process. Here is a group of organisms that can oxidize ammonia completely to N_2 in one tank and require nothing but anoxia and a supply of CO_2. In fact, anammox was discovered in an enrichment culture from a wastewater treatment plant, and the real mystery is why it was not found

before. This may be due to its slow growth rate; many water treatment plants may have discouraged the development of the anammox process by the timing and conditions of treatment stages. Anammox bacteria have never been grown in pure culture; they apparently require the presence of complex consortia including nitrifiers and or denitrifiers, in order to obtain both ammonium and nitrite in the right proportions. Commercial anammox reactors for wastewater treatment usually involve a mixture of aerobic nitrifiers and anammox bacteria. The nitrifiers produce the nitrite required for anammox, and consume trace oxygen that would inhibit the anammox reaction. Establishment of a stable reactor consortium can require years (Kuenen, 2008).

The Marine Environment

The nitrogen cycle of the ocean is interesting because of the role of N as a limiting nutrient for primary production in the sea, and because the ocean is the ultimate repository for waste from land, in the form of wastewater effluent and natural drainage from rivers. Nitrogen loading in natural waters, from excess fertilizer applications as well as wastewater effluent, has increased in recent decades, such that the impact in coastal waters is now detectable. Nitrification plays a part in both of these processes as described above.

The Ocean Ecosystem

The two primary forms of nitrogen that are available to phytoplankton as nutrients are the same as those used by terrestrial plants, ammonium and nitrate. Ammonium is supplied to surface waters via recycling of organic nitrogen in waste products of grazers and heterotrophic bacteria. It is rapidly recycled in the euphotic zone (well lit surface layer of the ocean) and usually present in very low concentrations. Nitrate is supplied to the sunlit surface waters by upwelling or wind mixing of deeper waters where nitrate concentrations are generally elevated, or by nitrification of ammonium in near surface waters. Although both ammonium and nitrate are suitable N sources for many phytoplankton, different species of phytoplankton exhibit important differences in their abilities to utilize and grow on them. For example, most clades of the most abundant small phytoplankton, a small cyanobacterium called *Prochlorococcus*, cannot grow on nitrate at all, and some forms cannot utilize nitrite either. Their only inorganic N source is ammonium. *Prochlorococcus* is most important in the oligotrophic central gyres of the oceans, where the mixed layer is so deep that nitrate is rarely mixed into the photic zone; natural selection has evidently led to the loss of genes involved in the assimilation of oxidized nitrogen because they were not useful in this environment.

At the opposite extreme are diatoms, eukaryotic phytoplankton with silicious shells, which are extremely important in upwelling and coastal regimes where nitrate is typically more abundant. Diatoms often show a strong preference for ammonium, presumably because it requires less energy to assimilate than does nitrate. This preference is demonstrated by the observation that in the presence of both ammonium and nitrate, even when the latter is present at much higher concentrations, diatoms will first use up the ammonium before beginning to assimilate nitrate. The irony is, however, that their subsequent growth on nitrate can be much faster than the earlier growth on ammonium. Diatoms can attain very high growth rates on nitrate, and are characteristic bloom formers because they can grow much faster than their grazers can, and thus they avoid predation.

The significance of nitrification in this surface ocean N cycling is the conversion between ammonium and nitrate. The deep ocean has very high nitrate concentrations, while the surface ocean is usually quite depleted in nitrate, and this observation led to the long-held belief that nitrification occurred only in the deep sea. If that were true, then nitrate availability would be controlled by physical processes that somehow mix the deep water up into the sunlit surface zone. In fact, broad patterns of oceanic primary production can be explained at first pass by a consideration of the physical oceanographic constraints on mixing in various regions of the world ocean. It is now known, however, that AOB, AOA and NOB are present in the surface ocean and that the rate of nitrification shows a distinct maximum near the bottom of the euphotic zone. It can be shown in culture, enrichment experiments, and incubations of natural seawater that nitrification, both ammonia and nitrite oxidation, is inhibited by strong light, an observation that likely contributes to the depth distribution of nitrification. Even with maximum nitrification rates near the bottom of the euphotic zone (e.g., at a depth where 5–10% of the surface light intensity penetrates), nitrification can still supply much of the nitrate demand by primary producers. In this situation, nitrate is cycled rapidly too, and contributes to the support of primary production even when present at low levels. The depth distribution of nitrification rates is characterized by a subsurface maximum near the bottom of the euphotic zone, a rapid decrease in rate with increasing depth, and very low rates in the deep ocean (Ward, 2008). The accumulation of nitrate in deep waters is thus explained by its production at very low rates by nitrification and the very slow loss rates; that is, absence of phytoplankton N demand.

The bottom of the euphotic zone is often characterized by a primary nitrite maximum, a small but distinct accumulation of nitrite that usually occurs around the depth of 1% surface light penetration. The origin of this feature has long been debated, and the two main processes thought to be involved are nitrification and nitrate assimilation by phytoplankton. Light plays an important role in both proposed mechanisms. In the case of nitrification, it is proposed that NOB are more sensitive to light inhibition than are the ammonia oxidizers, such that the AOB or AOA are active at slightly more shallow depths than are the NOB (Olson, 1981). Thus, there is a net production of nitrite from ammonium oxidation, but at slightly deeper depths, the NOB are active and remove the nitrite. This is an attractive explanation and is consistent with the widespread occurrence of the primary nitrite maximum at essentially the same relative depth in many parts of the ocean. The alternative explanation is that phytoplankton involved in assimilation of nitrate find themselves severely light, and therefore energy, limited at this depth (Lomas and Lipschultz, 2006). After taking up nitrate, they are unable to obtain the reducing power necessary for its complete reduction to

ammonium for incorporation into biomass, and release some of it as nitrite. Both of these mechanisms might result in diel, as well as seasonal, variability in the feature. Both processes probably contribute the primary nitrite maximum, and their relative contributions vary with system and season.

The distributions of AOB, AOA and NOB are now much more well known due to recent advances in enumeration technology. AOA are generally more abundant than AOB and often show a depth distribution that is closely correlated with distribution of ammonium oxidation rate; i.e., low numbers in surface waters, a discrete subsurface maximum and decreasing numbers with depth. The depth distribution of NOB has been investigated very rarely; NOB are generally less abundant that AOA and likely have a similar depth distribution. Isotopic methods for measurement of nitrification rates are not dependent upon the kind of organism responsible for the process, and they generally show that rates decrease rapidly with increasing depth. This is consistent with the general pattern of decreasing biological activity overall with increasing distance from the surface layer.

Oxygen-Depleted Waters and Sediments

In addition to its role in controlling the nitrate distribution in the ocean, nitrification performs the same role in the ocean as it does in agriculture and wastewater treatment, in linking ammonium regeneration to denitrification and thus facilitating the conversion of organic N to N_2 (see Denitrification). In the ocean, denitrification is restricted to sediments and to a few regions of the water column where organic supply and ocean circulation cooperate to limit oxygen concentrations to very low levels. Three such regions account for essentially all of the water column denitrification in the ocean: the eastern tropical North Pacific (the Mexican Margin), the eastern tropical South Pacific (the Peru upwelling region), and the Arabian Sea (Devol, 2008). These regions are characterized by high surface productivity and limited intermediate water renewal, so that water in the depth interval of about 80–1000 m is very low in oxygen. In this interval, oxygen concentration is low enough that denitrification can occur, and nitrifier denitrification is also enhanced, leading to N_2O production. It is not known how much nitrification and denitrification each contribute to the N_2O flux, but these oceanic regions are responsible for most of the atmospheric N_2O flux from the ocean.

As in other regions of the ocean, nitrification rates are generally highest in the near subsurface region of oxygen minimum zones (OMZs), where there is usually a strong primary nitrite maximum. OMZs are also characterized by the presence of a strong secondary nitrite maximum (Figure 1), usually at the heart of the low-oxygen depth interval where it is assumed that oxygen concentrations are too low to support conventional aerobic nitrification. Nitrite concentration in the secondary nitrite maximum can be much higher than found in the primary nitrite maximum, and is thought to derive from partial denitrification, although an adequate mechanism has never been proved.

Nitrous oxide also has a characteristic distribution in OMZs (Figure 1), typically exhibiting two maxima; one occurs just below the primary nitrite maximum and is often attributed to nitrifier denitrification. The second N_2O maximum occurs below the secondary nitrite maximum and is usually attributed to denitrification. At the depth interval of the secondary nitrite maximum itself, N_2O is at a minimum, and its undersaturation here is attributed to complete denitrification. Although the main processes

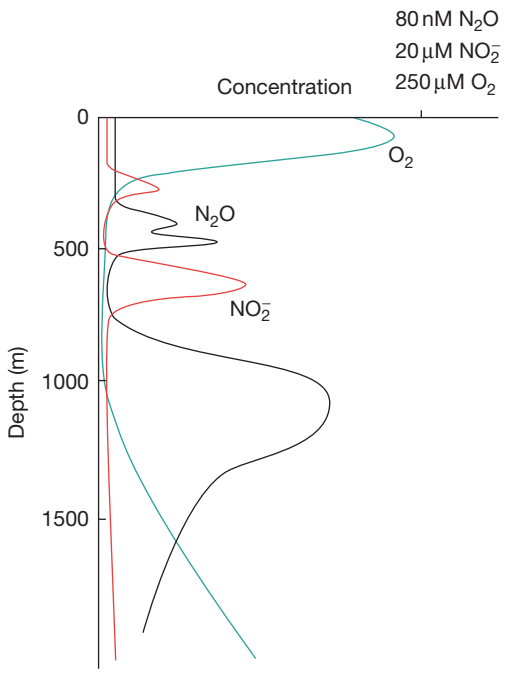

Figure 1 Characteristic distributions of nitrite (NO_2^-) nitrous oxide (N_2O) and oxygen (O_2) in the water column of an oxygen minimum zone (OMZ) in the ocean. Concentration axis shows the scale for each plotted variable.

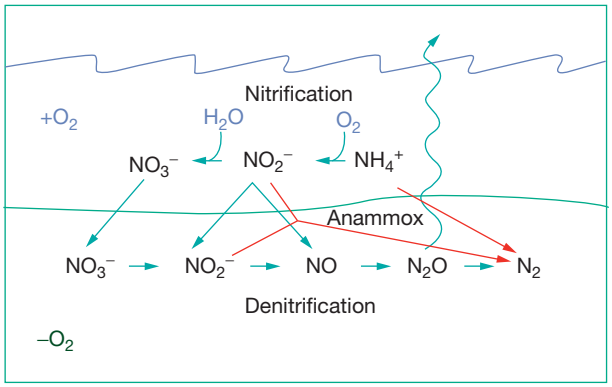

Figure 2 Interactions between nitrification, denitrification, and anammox across the oxic ($+O_2$)/anoxic ($-O_2$) interface in a marine sediment. In the oxic layer (above the green line) nitrification oxidizes ammonium to nitrate. Nitrate and nitrite diffuse into the anoxic layer where they can be denitrified or used in combination with ammonium for anammox. Both denitrification and anammox are ultimately dependent upon nitrification for the production of oxidized N species, which are reduced to N_2 gas.

involved in the maintenance of these characteristic distributions are probably known (i.e., nitrification, denitrification, anammox), the mechanisms by which they are regulated to result in such predictable distributions is unknown.

Anammox does not produce or consume N_2O but can be an important component of the total fixed N loss in OMZ regions, because it contributes to the production of N_2. On a global basis, its contribution to fixed N loss is regulated by the stoichiometry of organic matter that is degraded in the OMZs and averages about 29%, with denitrification responsible for the rest (Dalsgaard et al., 2012).

Coastal regions that receive nitrogen-rich runoff from land are susceptible to nutrient enrichment leading to eutrophication. This can also lead to low-oxygen conditions in the shallow bottom water, and thence to the production of N_2O and fixed N loss via denitrification and anammox, as described for the low-oxygen oceanic regimes. Both coastal and oceanic low-oxygen regions are likely to be sensitive to environmental change brought about by changes in nutrient loading and stratification, both factors that respond to anthropogenic forcing of the atmosphere and land systems. Denitrification, nitrification, and anammox are all sensitive to oxygen concentration, so the extent of oxygen-depleted waters may have a major effect on the overall N budget of the oceans.

Coupled nitrification/denitrification and anammox are also very important in the N cycle of marine sediments. Approximately half the N_2 production via anaerobic organic matter degradation is derived from anoxic sediments, where degradation by aerobic heterotrophs of organic matter settling down from overlying waters leads to consumption of oxygen in the sediments. Ammonium released from both aerobic and anaerobic decomposition of organic matter diffuses into the oxygenated zone of surface sediments and is nitrified to nitrate. Nitrification is often identified as the main sink term for oxygen in oceanic sediments. Conventional nitrification can be coupled to denitrification by the diffusion of intermediates and end products across the oxic/anoxic gradient near the surface sediment. Similarly, anammox is dependent upon supply of oxidants (nitrate or nitrite) that are ultimately derived from conventional nitrification (**Figure 2**). Both conventional nitrification, coupled to denitrification, and anammox are important in sediment systems, and because of the metabolic differences (especially their relationships to oxygen and organic matter) among the organisms responsible, it is likely that their contributions vary greatly among estuarine, shallow, and deep marine systems.

Environmental Factors that Affect Nitrification

Several environmental factors that might control nitrification in various ecosystems have already been mentioned. They include the kinds of things that affect biological processes in general, as well as those particular to the metabolism of nitrifiers: temperature, salinity, light, organic matter concentrations, substrate (ammonium and nitrite) concentrations, pH, and oxygen concentration. A few of the interesting and unique interactions of nitrifiers with their environment are explored below.

Ammonium, the primary substrate for AOB and AOA, is rarely abundant in oxic environments, so the rate of nitrification is likely to be at least partially controlled by substrate limitation. Most cultivated AOB have affinities for ammonium that preclude their being effective competitors for ammonium in the ocean. The one cultivated marine AOA strain, however, has a very high affinity for ammonium (Martens-Habbena et al., 2009), as do natural assemblages (Newell et al., 2013). The absence of ammonium in the deep ocean, where sinking organic material is mineralized to produce ammonium, speaks to the efficiency with which AOA, and to a lesser extent AOB, remove it.

AOB, AOA and NOB require molecular oxygen for their metabolism and thus are restricted to oxic environments. Nonetheless, they seem to prefer and to do quite well under very low oxygen conditions, displaying a microaerophilic lifestyle. Anammox organisms, in contrast, are strictly anaerobic, and while oxygen apparently does not kill them, it does inhibit their activity. Thus, oxygen concentration, in the bulk water of aquatic environments and in the interstices of sediments and soils, is likely a very important variable for regulation of microbial activities and the resulting distribution of nitrogen-cycling processes.

All of the nitrifying microorganisms are predominantly autotrophs, that is, they fix their own carbon from CO_2, and thus do not rely on a supply of organic matter for nutrition. This means that they are not in competition with heterotrophs for the utilization of organic substrates, but rather that they exploit a different niche. This niche involves certain 'sacrifices', in terms of slower growth rates (see Units of Selection). These forms of autotrophic growth are also quite inefficient, due to the low energy yield of the transformations involved. Thus nitrifiers process large amounts of nitrogen in order to obtain the energy required for CO_2 fixation. The molar ratio of N oxidized to C fixed has been estimated at 25–100 for AOB, AOA and NOB, ensuring that their metabolism has a very large effect on the nitrogen cycle, but very little influence on the carbon cycle, where photosynthetic autotrophs are overwhelmingly important.

Light inhibition of nitrifiers is suspected as a mechanism for the formation of the primary nitrite maximum (see above) and it is easily demonstrated in culture that both AOB and NOB are sensitive to light. Cultivated AOA are at least as light sensitive as AOB, perhaps even more so (Merbt et al., 2011). The basis for the light sensitivity of AOB and NOB is assumed to be damage to the many cytochromes that are involved in the energy transduction pathways of nitrification. AOA do contain cytochromes, so light sensitivity must be yet another way in which AOA and AOB exhibit similar physiologies but for different reasons.

Any transformation that involves the production or consumption of hydrogen ions is pH sensitive, and ammonia oxidation is no exception (see Acidification). Oxidation of ammonia by AOB and AOA results in the acidification of the medium. Low pH eventually inhibits both groups in culture, and activity can be restored by pH adjustment. Ammonia oxidation rates in the ocean were reduced by increased acidification, suggesting that nitrification might be affected by ocean acidification due to increased CO_2 concentrations in the atmosphere and ocean (Beman et al., 2011). Short term experiments, however, may overestimate the response to lowered pH, so the sensitivity of nitrification to long term global change is unknown. It is unlikely that pH is an important controlling variable in the ocean, even in sediments, but pH could be very important in regulation of nitrification in acid soils. While nitrification generally occurs in acid soils, it has proven difficult to obtain acidophilic nitrifiers in culture, leading to speculation about the importance of heterotrophic nitrification in this system. It is now known that many of the kinds of nitrifying bacteria that can be identified by their gene sequences in the natural environment, are not in fact represented in culture collections. Thus it is quite possible that acidophilic autotrophic nitrifiers exist but are resistant to cultivation.

Salinity and temperature do not appear to set any unusual constraints on the range of conditions under which nitrification can occur, and different kinds of nitrifiers appear to have adapted to the wide range of these variables found on Earth. The mechanism by which salinity affects nitrification is not known, but it is clear that salinity is an important determinant of the community composition of nitrifying microbes, if not the net rate of nitrification; that is, different kinds of nitrifiers are adapted to different salinity levels, but nitrification occurs under high as well as low salinity and depends on the presence of different species. Nevertheless, it can be shown that salinity dramatically affects the rate of nitrification, when salinity changes are imposed in an experiment with natural assemblages. Ionic strength effects related to the sorption and availability of ammonium are not sufficient to explain the effects of salinity.

Methods for Measurement of Nitrification Rates

^{15}N Tracer Methods

The best method for quantification of nitrification rates remains the direct ^{15}N tracer approach in which a small amount of either $^{15}NH_4^+$ or $^{15}NO_2^-$ is added to a sample and incubated (Ward, 2010). The labeled product, $^{15}NO_2^-$ or $^{15}NO_3^-$, is then extracted and measured on a mass spectrometer. This approach has suffered in the past from the necessity to enrich the substrate pool by the addition of large concentrations of ^{15}N-ammonia or -nitrite, thus artificially enhancing the observed rate. Improvements in assay techniques and mass spectrometer sensitivity with small N masses have minimized this problem.

The same analytical approaches can be applied in a tracer or isotope dilution format. In the isotope dilution format, label is added to the product pool and its dilution by addition of new product with natural abundance isotopic signature during the incubation provides an estimate of production rate.

The advantages of the direct ^{15}N approaches, compared to inhibitor methods, include shorter, thus less artifactual, incubations, minimal perturbations to *in situ* conditions (ambient light and nutrient conditions can be used), and much greater sensitivity. Inhibitor-based assays have the advantage, however, of requiring simpler less expensive instrumentation, as colorimetric, rather than mass spectrometric, analysis usually suffices.

Inhibitor-Based Nitrification Assays

Inhibitor methods depend on the ability of many compounds to interact specifically with the active site of the ammonia monooxygenase (AMO) or nitrite oxidoreductase (NXR) enzyme. A large number of potential inhibitors has been used for AMO, while chlorate is still the only specific inhibitor reported for nitrite oxidation. Replicate incubations are carried out in the dark with additions of either AMO or NXR inhibitors and the changes in inorganic N concentrations are used to infer nitrification rates. A more sensitive permutation of this approach involves measurement of $^{14}CO_2$ fixation in the presence and absence of inhibitor, where the decrease in the rate of $^{14}CO_2$ assimilation in the presence of nitrifier inhibitor is attributed to nitrification (Rees et al., 2002). A conversion between CO_2 fixation and N oxidation rates is then used to estimate nitrification.

One of the main attractions of the inhibitor approaches is their ease of use and analysis. Scintillation counters are much more common and easier to use than instruments required for stable isotope analysis and inorganic N determinations involve analytical methods that are already standard to most laboratories. Although the inhibitor approaches are usually not appropriate for absolute rate measurements (because of uncertainty in conversion factors and perturbations during incubations) they can be very useful for spatial or temporal comparisons within studies.

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